FUNGI ACTIVE IN HETEROTROPHIC NITRIFICATION[1]

K. C. MARSHALL[2] AND M. ALEXANDER

The production of nitrite and nitrate from peptone was described first by Schmidt (7) in the fungus *Aspergillus flavus*, and subsequent studies have revealed that many strains of *A. flavus* are capable of heterotrophic nitrification (2, 3, 4). In addition, Malavolta *et al.* (4) have shown that a culture of *Aspergillus wentii* was able to form nitrite and nitrate in a peptone medium while 10 isolates of *Aspergillus niger* and 2 of *Aspergillus oryzae* were unable to carry out the oxidation. Nitrate production in a medium containing nitrite as a sole nitrogen source has been described in a number of aspergilli (6), in a strain of *A. flavus*, and in several species of *Penicillium* (3).

In an attempt to extend the range of heterotrophic nitrifying fungi, cultures obtained from various sources were grown in the peptone medium described by Eylar and Schmidt (2) and in an ammonium medium made up of two parts. Part A contained sucrose, 8.8 g; $(NH_4)_2HPO_4$, 1.65 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2.2H_2O$, 0.1 g; $FeSO_4.7H_2O$, 0.001 g; $MnSO_4.4H_2O$, 0.001 g; and distilled water, 500 ml. Part B contained K_2HPO_4, 0.1 g; $Na_2HPO_4.7H_2O$, 26.8 g; NaH_2PO_4, 13.9 g; and distilled water, 500 ml. The two solutions were autoclaved separately to avoid excessive precipitation and were mixed after being allowed to cool. The microorganisms were incubated at 30° C in a static condition. Since cultures of *A. flavus* tend to lose the ability to nitrify after prolonged culture on artificial media (8), all the fungi were inoculated into sterile soil immediately upon receipt and reisolated prior to use.

[1]Supported in part by a grant from the National Science Foundation (6-8960) and an Overseas Studentship awarded to the senior author by the C.S.I.R.O., Australia.

[2]Present address: Division of Soils, C.S.I.R.O., Nedlands, Western Australia.

TABLE I

Production of nitrite and nitrate by various fungi in peptone and ammonium media

Identity	Culture No.*	Incubation time (days)	Oxidized nitrogen formed (μg N/ml)			
			Peptone medium		Ammonium medium	
			Nitrite	Nitrate	Nitrite	Nitrate
Aspergillus flavus	ATCC1003	20	0.09	0	—	—
A. flavus	ATCC9296	20	0.33	13.0	—	—
A. flavus	ATCC10124	20	0.35	24.5	—	—
A. flavus	ATCC11495	20	0.06	0	—	—
A. flavus	NRRL1779	6	0	0	0	0
A. flavus	NRRL1819	6	0.18	38.5	0	29.5
A. flavus	IZ (15)	6	0	0	0.05	34.5
A. flavus	IZ (1219)	6	0.24	9.8	0.07	25.0
A. flavus	Schmidt	6	0.56	10.5	0.06	40.0
A. flavus	Bush	6	0	0	0	0
Aspergillus oryzae	ATCC9102	20	0.18	4.5	—	—
A. oryzae	NRRL1809	6	0.95	7.0	0.06	9.5
A. oryzae	NRRL1988	6	0	0	0	0
Aspergillus wentii	NRRL1599	6	0	0	0.12	34.5
A. wentii	NRRL1778	6	0	0	0.08	8.0
A. wentii	IZ (1220)	6	1.20	0	0	25.5
Penicillium atrovenetum	SM 683	6	0.23	0	0.10	0

*Origin of cultures

ATCC: American Type Culture Collection, Washington, D.C.
NRRL: Northern Utilization Research and Development Division, U.S.D.A., Peoria, Illinois.
IZ: Dr. J. D. P. Arzolla, Instituto Zimotécnico, Piricicaba, Brazil.
Schmidt: Dr. E. L. Schmidt, University of Minnesota, Minneapolis.
Bush: Dr. M. T. Bush, Vanderbilt University, Nashville, Tennessee.
SM683: Dr. J. H. Birkinshaw, London School of Hygiene and Tropical Medicine.

The results in Table I provide further evidence for the widespread occurrence of the nitrifying ability among strains of *A. flavus*, and indicate that this property may be common to strains of *A. wentii*. It is obvious that some cultures of *A. oryzae* also are capable of oxidizing reduced forms of nitrogen to nitrite and nitrate.

Since β-nitropropionic acid has been implicated in the pathway of heterotrophic nitrification by Schmidt's strain of *A. flavus* (Marshall and Alexander, unpublished data), two fungal cultures known to be able to produce β-nitropropionic acid under certain conditions were obtained and tested for their ability to produce nitrite or nitrate in the media described above. The *A. flavus* strain of Bush *et al.* (1) failed to produce nitrite or nitrate while *Penicillium atrovenetum* SM683 (5) formed small quantities of nitrite but no nitrate in both media. Surprisingly, this culture of *P. atrovenetum* did produce significant amounts of nitrate (13 μg nitrate-N/ml) when grown in the nitrite medium suggested by Hora and Iyengar (3). The ATCC and NRRL cultures listed in Table I were grown in this nitrite medium, but only two strains of *Aspergillus wentii* (NRRL 1599 and NRRL 1778) accumulated nitrate.

1. BUSH, M. T., TOUSTER, O., and BROCKMAN, J. E. The production of β-nitropropionic acid by a strain of *Aspergillus flavus*. J. Biol. Chem. **188**, 685–693 (1951).
2. EYLAR, O. R. and SCHMIDT, E. L. A survey of heterotrophic microorganisms from soil for ability to form nitrite and nitrate. J. Gen. Microbiol. **20**, 473–481 (1959).

3. HORA, T. S. and IYENGAR, M. R. S. Nitrification by soil fungi. Arch. Mikrobiol. **35**, 252–257 (1960).
4. MALAVOLTA, E., de CAMARGO, R., and HAAG, H. P. Nota sobre a nitrificacao por fungos do solo. Bol. inst. zimotéc. (São Paulo), No. **13** (1955).
5. RAISTRICK, H. and STOSSL, A. Studies in the biochemistry of microorganisms. 104. Metabolites of *Penicillium atrovenetum*. G. Smith: β-nitropropionic acid, a major metabolite. Biochem. J. **68**, 647–653 (1958).
6. SAKAGUCHI, K. and WANG, Y. Studies on the assimilation of nitrites by fungi. Part II. Bull. Agric. Chem. Soc. Japan, **12**, 63–69 (1935).
7. SCHMIDT, E. L. Nitrate formation by a soil fungus. Science, **119**, 187–189 (1954).
8. SCHMIDT, E. L. Cultural conditions influencing nitrate formation by *Aspergillus flavus*. J. Bacteriol. **79**, 553–557 (1960).

RECEIVED JULY 24, 1961.
LABORATORY OF SOIL MICROBIOLOGY,
DEPARTMENT OF AGRONOMY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK.